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Phospholipids in Algae for Biodiesel Production

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Introduction

Biodiesel producers have long recognized the need for new sources of oil feedstock if biodiesel is to continue displacing petroleum-based diesel fuel. One potential source of oil that could minimize competition with food crops is microbial oil, with photosynthetic algae being the most widely studied option. An understanding of algal lipids, their native biochemistry, and interactions once extracted, is essential to the process of learning to make the production of biofuels from algal oils more efficient and economically viable. An issue with the use of algal oils is that they frequently contain large amounts of phospholipids, which are essential components of cell walls and may be extracted from solid biomass when the oil is separated [1].

Phospholipids, also called phosphatides or gums, have structures that are similar to the triglycerides that compose the largest fraction of most algal oils. Whereas triglycerides consist of fatty acid chains attached to each of the three carbon atoms of a glycerin backbone, phospholipids have only two fatty acid chains and the third carbon atom is attached to a collection of atoms that includes phosphorus. The configuration of the phosphorus group determines the type of phospholipid and the most common groups have names like phosphatidylcholine, phosphatidic acid, and phosphatidylethanolamine. The phospholipids are typically present in terrestrial vegetable oils at concentrations of 2 to 5% and although they are not toxic, they tend to form insoluble precipitates with water. Because of this, they are removed from food-grade oils to prevent the formation of an unappetizing scum during cooking. Phospholipids that are present in biodiesel may plug fuel filters, create engine deposits, and damage exhaust after- treatment devices.

When water is absorbed into extracted oils, hydration of phospholipids occurs, which causes these lipids to become insoluble. These lipids are commonly referred to as gums. Typically, these gums need to be removed prior to transesterification, commonly by warm water washing. Water washing creates its own issues with treatment and disposal of the generated wastes, which can amount to 0.2 tons per ton of fuel produced [2]. Some phospholipids are not removed by water washing and must be treated with acid, usually phosphoric or citric, before they are hydrated and will precipitate. According to Amoah et al. [1], degumming is not an attractive approach in microalgal lipids due to the presence of higher levels of phospholipids and the amount of useful feedstock that is lost in the process.

Transesterification is a necessary step in converting viscous plant and algal oils into something which can be reasonably used for fuel. The need to de-gum or otherwise avoid polar lipids prior to transesterification is both costly and time-consuming. The presence of phospholipids is known to reduce the conversion efficiency of plant derived oils to fatty acid methyl esters (FAME) [3,4]. However, the mechanisms of this interference have rarely been studied. Most of the work in this area has been conducted with crude plant oils and relates to the interference observed with immobilized lipases in enzymatically catalyzed transesterification processes (i.e., [4]). In these enzymatically catalyzed processes, it appears that the interference is the result of either phospholipid binding to the immobilized lipase and interfering with the interaction of the enzymes with their substrates [4] or glycerol binding to the underlying matrix upon which the lipases are immobilized [3], or glycerol binding the surface of the immobilized enzyme [5]. Amoah et al. [1] suggested that the presence of phospholipids allows for greater contact time between lipase and methanol, which leads to alcohol deactivation of the enzyme. The question still remains as to the mechanisms that inhibit transesterification in crude oil catalyzed with an alkali catalyst. It has been suggested that phospholipids contribute to the destruction of catalysts [6,7], however, the exact mode of this inhibition has yet to be explained.

Lipids and TAGs in Microalgae

Lipids in algae are produced in the chloroplasts via multiple pathways, including the direct glycerol pathway [8] and the Kennedy pathway [9]. Fatty acids are synthesized and then assembled into various polar and neutral lipids. Under normal growth conditions, synthesized phospholipids, glycolipids and other polar lipids, are primarily produced as cell wall and membrane components which constitute 5 to 20% of the organism's dry cell weight [8]. Along with associated proteins, phospholipids function both as structural elements as well as vehicles in transporting nutrients and metabolites across membranes [10]. Within cell walls and membranes, the structure and composition of phospholipids are thought to be responsible for cellular signaling, cell to cell interactions and for their roles in biological processes, such as stress response and photosynthesis [11]. Phospholipids are also the primary component of lipid oil body membranes within the cytoplasm [12].

Triglycerides are principally storage components for energy reserves. Produced in the chloroplasts, triglycerides are extruded into oil bodies, which are surrounded by an envelope of phospholipids and associated proteins [12]. Oil bodies are particularly stable both in vivo and when removed from their cellular environment [12,13]. When algal cells age or find themselves under environmental stress, such as nutrient deficiency, the chloroplasts will begin to break down, and cellular energy resources are redirected to the selective production of triglyceride molecules, which can make up 20 to 50% of their dry cell weight [8]. Oil body membranes, in addition to the phospholipids and structural proteins, also contain lipases and other lipid transforming enzymes, indicating the dynamic nature of lipids within cells. Triglycerides accumulated during times of stress can be rapidly converted back to structural and growth elements when environmental conditions improve [14].

Lipid Effect on Transesterification

Researchers have investigated different processes of transesterification in an attempt to avoid the need to de-gum, notably, lipase mediated transesterification and supercritical methanolysis [15]. Lipase transesterification avoids some of the issues that are encountered with alkali transesterification, namely that of the effects of free fatty acids and water in the reaction system, as well as the need to treat wastewater. However, lipases are expensive, and as stated above, still susceptible to interference from either phospholipids or glycerol in the transesterification process [3]. Cesarini et al. [3] have suggested a regime utilizing two phospholipases in combination with a transesterification lipase which appears to eliminate the phospholipid interference and the need to de-gum crude soybean oil.

Supercritical methanolysis also appears to be promising. In this process, FAME can be readily produced not only from triglycerides but also from polar lipids and free fatty acids [16]. This process also potentially eliminates the need for drying the algae and extracting the lipids prior to transesterification, which are time and energy intensive processes and amount to 90% of the cost of production [17,18]. Production cost via supercritical methods has been claimed to be half of that for conventional transesterification [16].

The latest research seems to indicate that phospholipids may act as a shield, attracting water that either prevents triglycerides from being accessed for reaction, or simply consumes catalyst, which decreases the amount available to drive the reaction [19]. Amoah et al. [1] indicated that phospholipids form reverse micelles around water droplets in extracted oils, also encapsulating methanol and catalyst. This limits the availability of these components for transesterification. Phospholipids interfere with the aqueous non-aqueous interface area and thus isolates and concentrates water, methanol and enzymes in reverse micelles [1]. Increasing the volume of methanol in the reaction decreases the ability of the phospholipids to envelop the water soluble elements, thus facilitating the amount of chemicals available to carry out the transesterification process. This would also explain why introducing phospholipases into the reaction system, as demonstrated by Cessarini et al. [3], can increase FAME production.

Summary

The question remains, where do we go from here to find an economically viable approach to producing biofuels from algal substrates. Phospholipids are elements that will need to be addressed, either by avoiding them, as in the lipase mediated processes and supercritical methanolysis, or by simply washing them away with the resulting loss of feedstock. The presence of phospholipids is intimately related to the presence of water in the process of producing FAME. Forming the interface between oil and water, phospholipids necessarily insert themselves into the equation. It may take life cycle analyses to determine the most efficient means of producing biofuels from this complex system. This needs creative thinking and innovative chemistry.

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